respect to the less stable mesophile. When focusing on different time- and length- scales specific behaviors arise. At an atomistic scale, it is found that in the hyperthermophile a more regular alternation of rigid and flexible regions stabilizes a key part of the protein where the unfolding of the mesophile begins. We furthermore find that the conformational landscape of the hyperthermophile is characterized by a higher number of substates, or otherwise an enhanced conformational flexibility that is suggested to broaden its stability curve and raise the melting temperature. We finally compare, for the two proteins, the unfolding paths upon increasing temperature, the kinetic barrier along the early steps of unfolding and the temperature dependency of the stability.

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1302-Pos Board B32

Exploring the Relation between Unfolded Protein Ensembles, Transformations between Structures, and Refolding Kinetics

Steven Samuel Plotkin.
University of British Columbia, Vancouver, BC, Canada.

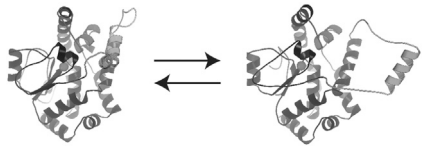
We develop a method for generating a diverse conformational ensemble, to characterize properties of the unfolded states of intrinsically disordered or intrinsically folded proteins, with or without disulfide bonds. We can thus examine physical properties of the unfolded ensembles for various proteins, including chemical shifts, residual dipolar couplings, clustering properties, and scaling exponents for the radius of gyration with polymer length. We apply our generated ensembles to the problem of folding kinetics, by examining whether the ensembles of some proteins are closer geometrically to their folded structures than others. We find that for a randomly selected dataset of 15 non-homologous 2- and 3-state proteins, quantities such as the average root mean squared deviation between the folded structure and unfolded ensemble correlate with folding rates as strongly as absolute contact order. We introduce a new order parameter that measures the distance travelled per residue, which naturally partitions into a smooth "laminar" and subsequent "turbulent" part of the trajectory. This latter conceptually simple measure with no fitting parameters predicts refolding rates with remarkable accuracy ($r = -0.95$, $p = 1e\text{-}7$). The high correlation between folding times and sterically modulated, reconfigurational motion supports the rapid collapse of proteins prior to the transition state as a generic feature in the folding of both two-state and multi-state proteins. This method for generating unfolded ensembles provides a powerful approach to address various questions in protein evolution, misfolding and aggregation, transient structures, and molten globule and disordered protein phases.

1303-Pos Board B33

Coupling between Protein Conformation and Local Unfolding Highlights the Role of Disorder in Protein Function and Suggests a New Target for Tuberculosis Treatment

Thomas E. Morrell[1], Ilona U. Rafalska-Metcalf[1], Jhih-Wei Chu[2], Haw Yang[1].
[1]Princeton University, Princeton, NJ, USA, [2]National Chiao Tung University, Hsinchu, Taiwan.

Mycobacterium tuberculosis protein tyrosine phosphatase B (PtpB), which plays a key role in tuberculosis virulence, protects its active site from oxidation through a large-amplitude conformational change. The proposed regulatory mechanism for this protective motion involves a local unfolding event. We quantitatively show that local unfolding transitions can be coupled to other conformational changes in proteins. Molecular dynamics simulations were used to determine the conformations of a disordered protein region and the energy landscape for local unfolding. The accuracy of this energy landscape was tested and confirmed experimentally, providing rigorous support for our simulation approaches. Our work demonstrates that local unfolding can depend on protein conformation by changes in the stability of the disordered region. These results provide a mechanism for the internal regulation of protein conformational changes, which highlights new targets for the development of tuberculosis therapeutics.



1304-Pos Board B34

Computational Methods for Measuring the Free Energy of Folding in the Ribosomal Exit Tunnel

Anthony Hazel, James C. Gumbart.
Georgia Institute of Technology, Atlanta, GA, USA.

As a protein is synthesized in the ribosome, the nascent peptide chain, starting from the peptidyl transferase center (PTC), elongates along the ribosomal exit tunnel, which is ~10-20Å in diameter and ~100Å long. It has been shown that proteins can partially fold inside the exit tunnel and that the ribosome can stabilize the native secondary structure of proteins. However, the mechanism for this stabilization is not yet known at the atomic scale. To determine this mechanism, one can contrast the free energy of α-helix formation in water and in the ribosomal exit tunnel using molecular dynamics (MD) simulations. To determine the free-energy landscapes in water, we employed two computational methods - umbrella sampling (US) and adaptive biasing forces (ABF) - on various polyalanine-containing peptides, using the end-to-end distance of the polyalanine sequence as our reaction coordinate. Since this reaction coordinate does not produce a 1-to-1 correspondence to helical content, successive constraints were added to the simulations, and the changes in the free energy upon addition of each set of constraints were examined. We also applied extended ABF using the helical content of the polyalanine sequence as a reaction and compared the results with the end-to-end distance coordinate. Finally, we used these computational methods to calculate the free-energy landscape along the entire ribosomal exit tunnel with polyalanine-containing sequences placed at different locations.

1305-Pos Board B35

Structure and Dynamics of Intermediate Protein States by NMR and Simulations

Alfonso De Simone.
Imperial College London, London, United Kingdom.

The detailed characterization of the structure and dynamics of proteins and peptides in solution is crucial for a comprehensive understanding of complex biophysical mechanisms. Backbone dynamics from nanoseconds to seconds allow proteins to explore high-energy conformational states playing key roles in many biological processes. Using novel interdisciplinary approaches combining NMR experiments and simulations we have been able to effectively study protein dynamics and structures, including those "invisible" high-energy states that cannot be characterized by classical approaches of structural biology. These methods have proved to be highly effective in characterizing elusive states such as transition states in enzymatic processing and intermediates along the pathways of amyloid formation.

1306-Pos Board B36

Elucidating the Structural Basis of α-Synuclein Fibrillation using Small Camelid Nanobodies

Farah El Turk, Giulia Tomba, Erwin De Genst, Tim Guillams, Predrag Kukic, Michele Vendruscolo, Christopher Dobson.
University of Cambridge, Cambridge, United Kingdom.

α-Synuclein has been widely accepted, since its discovery, as an intrinsically disordered protein that plays a central role in Parkinson's disease, as well as other neurodegenerative disorders associated with protein aggregation. Extensive recent data substantiate the pathogenicity of the early aggregates of α-synuclein, rather than the characteristic amyloid fibrils observed in the late stages of the aggregation process. Therefore, understanding the molecular steps and the mechanisms by which this natively unfolded protein aggregates is crucial for the purpose of identifying novel diagnostic and therapeutic strategies for the treatment of synucleinopathies. A powerful therapeutic approach is to target the initial events in the reaction process, in order to promote the solubility of the monomeric form of α-synuclein and prevent the formation of potentially harmful assemblies. Thus, in our study, we aim at understanding the structural properties of the monomer that determines its aggregation propensity, using nanobodies, the antigen-binding domains derived from camel heavy chain antibodies. These molecules are valuable probes for elucidating whether conformational changes in the monomeric protein cause the aggregation, as result of their exquisite specificity, high affinity and small size (14 KDa). Our strategy is based on the study of the interactions between α-synuclein and two specific nanobodies that bind to its C-terminus and modulate its fibrillation. The structure and dynamics of α-synuclein in its free and bound states are characterized via a combination of NMR spectroscopy and in silico tools. More specifically, chemical shifts measurements, RDCs and restrained Molecular Dynamic simulations are applied to provide a comprehensive energy sampling and description of the conformational ensemble populated by α-synuclein, and thus help gain detailed insight into the mechanism by which nanobodies modulate the aggregation process of α-synuclein.

1307-Pos Board B37

Influence of Gold Nanoparticles on the Kinetics of Alpha-Synuclein Aggregation

Yanina D. Alvarez[1], **Jonathan A. Fauerbach**[1], Jessica V. Pellegrotti[1], Thomas M. Jovin[2], Elizabeth A. Jares-Erijman[1], Fernando D. Stefani[1].
[1]University of Buenos Aires, Buenos Aires, Argentina, [2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Alpha-synuclein (AS) is a presynaptic protein lacking a unique secondary structure in solution. AS amyloid aggregates in dopaminergic neurons are the